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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NEW METAL COMPLEXES WITH OROTIC ACID

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ABSTRACT

The aim of the present work is to synthesize and characterize new metal complexes. Novel pseudohalide ligands with orotic acid and its metal salt Ni(II) have been synthesized and evaluated for their antimicrobial activities by disc diffusion method. The complexes have been characterized by IR and UV-Visible spectroscopic techniques. The Diaquabis (imidazole) orotatoNickel(II), $[Ni(HOr)(H_2O)_2(Imd)_2]$(1), Diazidobis (orotato) nickel(II)], $[Ni(HOr)_2(N_3)_2]$(2), Diisocyanatobis(orotato) nickel(II)], $[Ni(HOr)_2(NCO)_2]$ and Dithiocyanatobis (orotato) nickel(II)] ,$[Ni(HOr)_2(NCS)_2]$ have been synthesized and characterized by means of elemental analysis, IR, UV–Vis studies. The Ni(II) ions in $[M(C_5H_2N_2O_4)(H_2O)_2(C_3H_4N_2)_2]$ the complex has a distorted octahedral coordination geometry comprised of one deprotonated pyrimidine N atom and the adjacent carboxylate O atom of the orotate ligand, two tertiary imidazole N atoms and two aqua ligands.

KEYWORDS: Antimicrobial Activity, Complex, Ligand, Orotic Acid

INTRODUCTION

Transition metal complexes of orotic acid and its mixed ligand derivatives continue to attract attention because of orotic acid's multidentate functionality and its considerable role in bioinorganic chemistry[1]. Metal orotates are also widely applied in medicine[2]. In addition, platinum, palladium and nickel orotates with wide variety of substituents have been screened as therapeutic agents for cancer[3]. Orotic acid (Vitamin B13, uracil carboxylic acid) plays a key role in the biosynthesis of pyrimidine bases of nucleic acids[4]. Some metal compounds of the acid itself and derivatives have successful applications in curing syndromes related with metal ion deficiencies[5] and promising applications as therapeutic agents for cancer [6]. The biocoordination chemistry of vitamin B13 therefore demands a better understanding regarding its interactions with metal ions. The orotic acid molecule (H_3Or) has a multidentate nature, The most potential coordination sites in the pH range of 3 to 9 are the carboxylic oxygen and the adjacent pyrimidine nitrogen atom (N1), for the formation of a stable chelate ring. In very alkaline solutions, deprotonation occurs from (N1) and coordination through the other sites becomes available as well due to the existence of different tautomeric forms [7-9] and the references therein. The literature lists several reports on mononuclear and polynuclear orotato complexes. Recent interest has focused on the proposed biological carrier function of orotic acid and the corresponding anionic species for metal ions, which is held responsible for the obviously successful application of metal orotates in curing syndromes associated with a deficiency of a variety of metals such as calcium, magnesium, zinc or iron [10,11].

EXPERIMENTAL

Reagents

All chemicals were purchased from Ranbaxy chemicals and used without further purification.

Synthesis

Synthesis of [Ni (HOr) $(H_2O)_2(imd)_2$] (1)

An methanolic (5 ml) solution of Nickel nitrate (0.291g, 1.0 mmol) was added to an sodium hydroxide solution(10ml) of Orotic acid under stirring conditions and the solution turned to sky blue with little turbidity and then aqueous solution (5 ml) of imidazole (0.068 g, 1.0 mmol) was added which resulted into a clear greenish blue solution. After constant stirring at room temperature for 30 minutes, The clear solution was filtered off and the solution is left for slow evaporation in the beaker and blue crystals were obtained in 2 weeks. The crystals were washed with methanol. They were soluble in methanol. Yield 0.190 g (0.652 mmol, 65.29 %). Anal. exptal. C_{12} H_{14} N_5 Ni O_6 (M.Wt. 382.99) C, 32.84; H, 4.110; N, 23.78. theoretical: C, 32.33; H, 3.81; N, 23.24. Important IR absorptions (KBr disk, cm^{-1}): 3445, 3227, 3137, 2614, 1620, 1559, 1491, 1260, 1097, 760, 746, 588 and 479, 454.

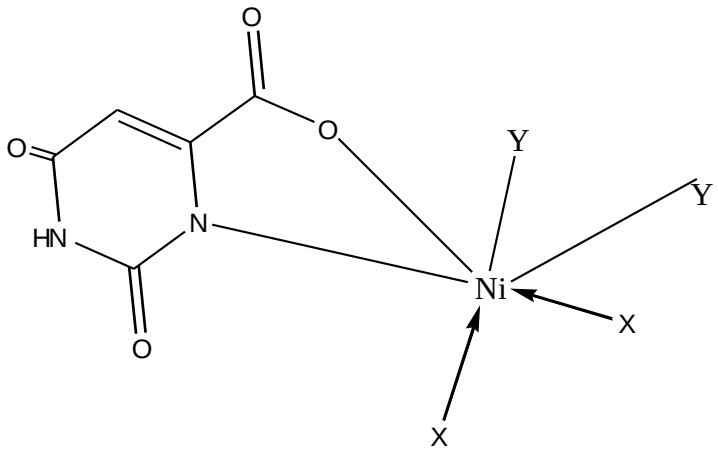

Figure 1: X = H_2O; Y = Imidazole

Synthesis of [Ni$(HOr)_2(N_3)_2$] (2)

An methanolic (5 ml) solution of Nickel nitrate (0.291g, 1.0 mmol) was added to an sodium hydroxide solution (10ml) of Orotic acid under stirring conditions and the solution turned to sky blue with little turbidity and then aqueous solution (5 ml) of NaN_3 (0.065 g, 1.0 mmol) was added which remained as same solution. After constant stirring at room temperature for 30 minutes,The solution was filtered off and the green gel was formed and the solution is left for slow evaporation in the beaker and green crystalline precipitate is obtained in one week. The crystalline precipitate was washed with methanol. It was soluble in methanol. Yield 0.180 g (0.618 mmol, 61.85 %). Anal. exptal. $C_{10}H_4N_{10}NiO_8$ (M.Wt. 450.89) C, 5.97; H, 2.45; N, 6.03. Found: C, 5.03; H, 2.38; N, 5.93. Important IR absorptions (KBr disk, cm^{-1}): 3545, 3522, 3441, 3417, 2140, 1635, 1473, 1382, 800, 634, 540 and 447.

Synthesis of [Ni$(HOr)_2(NCO)_2$] (3)

An methanolic (5 ml) solution of Nickel nitrate (0.291g, 1.0 mmol) was added to an sodium hydroxide solution (10ml) of Orotic acid under stirring conditions and the solution turned to sky blue with little turbidity and then aqueous solution (5 ml) of NaOCN (0.081 g, 1.0 mmol) was added which remained as same solution. After constant stirring at room temperature for 30 minutes, The solution was filtered off and the green gel was formed and the solution is left for slow evaporation in the beaker and green crystalline precipitate is obtained in one week. The crystalline precipitate was washed with methanol. It was soluble in methanol. Yield 0.160 g (0.55 mmol, 55 %). Anal. Cald. $C_{12}H_4N_6NiO_{10}$ (M.Wt. 450.89.) exptal: C, 8.64; H, 2.54; N, 4.35. Found: C, 6.73; H, 2.49; N, 3.95. Important IR absorptions (KBr disk, cm^{-1}): 3579, 3323, 2198, 2090, 1631, 1483, 1384, 1064, 786, 640, 543, and 451.

Synthesis of $[Ni(HOr)_2(NCS)_2]$ (4)

An methanolic (5 ml) solution of Nickel nitrate (0.291g, 1.0 mmol) was added to an sodium hydroxide solution (10ml) of Orotic acid under stirring conditions and the solution turned to sky blue with little turbidity and then aqueous solution(5 ml) of KNCS (0.097 g, 1.0 mmol) was added which remained as same solution. After constant stirring at room temperature for 30 minutes, The solution was filtered off and the blue gel was formed and the solution is left for slow evaporation in the beaker and crystalline precipitate is obtained in 3 days . The crystalline precipitate was washed with methanol. It was soluble in methanol. Yield 0.150 g (0.515 mmol, 51.5 %). Anal. Cald. $C_{24}H_{12}N_6NiO_2S_2$ (M.Wt. 481.89) C, 5.45; H, 2.31; N, 4.35. Found: C, 3.90; H, 2.28; N, 1.33. Important IR absorptions (KBr disk, cm^{-1}): 3443, 2064, 1642, 1486, 1384, 1166, 1060, 952, 650, 839, 477 and 459.

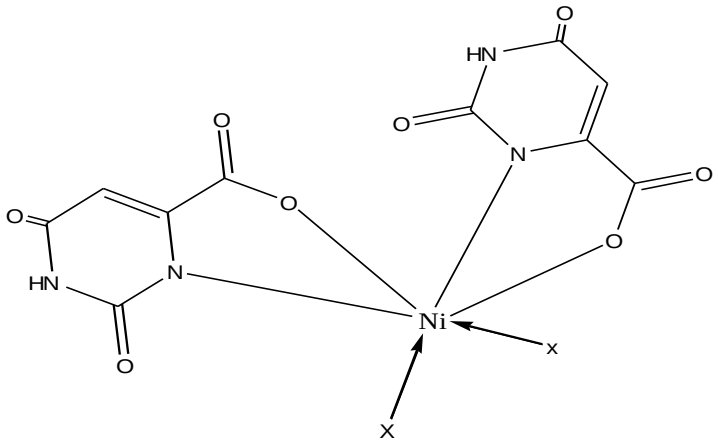

Figure 2: X = N_3, NCO, NCS

RESULTS AND DISCUSSIONS

Physical Properties

Table 1: Color, % Yield and Melting Point Data for the Complexes of the Orotic Acid

Compound	Color	% Yield	M.P.
L	White	-	Above 300^0C
$Ni(L)(Imd)_2(H_2O)_2$	Blue	65%	Above 300^0C
$Ni(L)_{2(}N_3)_2$	Green	62%	Above 300^0C
$Ni(L)_{2(}NCO)_2$	Green	55%	Above 300^0C
$Ni(L)_{2(}NCS)_2$	Green	52%	Above 300^0C

L = Orotic acid

IR Spectra

To understand the mode of bonding in the prepared complexes it was necessary to investigate the infrared spectra of the complex compounds and compare it to that of the free ligand. In general, metal complexation may increase or decrease the vibration frequencies of the coordinated function groups depending on the strength of the interaction occurring between the metal ion and the σ-electrons of the function groups. The strong and broad absorption bands of m(OH) vibrations of H_2O in complexes is observed at 3443 cm^{-1}. IR spectra of the complexes exhibit a medium intensity and broad band in the 3272–3178 cm^{-1} region which can be attributed to the N–H stretching vibration. The relatively weak two close bands at 2752–2964 cm^{-1} are due to the m(CH) vibrations. The strong and broad bands appeared at 1642, 1486 and1384 cm^{-1} regions in this complex is ascribed to the asymmetric and symmetric stretching vibrations of the coordinated carboxylate groups of the orotate ligand, respectively.The coordination of pseudohalide to the metal is confirmed by the presence of peak at 2060-2140 cm $^{-1}$. Similar absorption values have already been reported earlier for Ni(II) and

Co(II)-orotate complexes with imidazole and its derivatives and the positions of these bands have been well described in recently reports. In IR spectra of the complexes, m(OHacid) band at 2500 cm^{-1} in the orotic acid completely disappeared and a new carboxylate band m(COO) appeared between 1486 and 1384 cm^{-1}, respectively, indicating that the carboxylate group participates in the coordination with the metal ions by deprotonation.

Table 2: Selected Characteristics IR Bands (4000 – 400 cm-1) of Orotic Acid Complexes

Compound	O-H	N-H	C-H	Coo			Pseudohalide	OH(Acid)
				Sym	asym			
L	3523	3111	2835	-	1422		-	2500
$Ni(L)(Imd)_2(H_2O)_2$	3445	3227	2964	1620	1491		-	-
$Ni(L)_{2(}N_3)_2$	3441	3272	2964	1635	1473	1382	2140	-
$Ni(L)_{2(}NCO)_2$	3579	3323	2964	1631	1483	1384	2090	-
$Ni(L)_{2(}NCS)_2$	3443	3178	2964	1642	1486	1384	2064	-

L = Orotic acid

Electronic Spectra

The spectra of the orotate complexes in DMSO are shown that There are two detected absorption bands at around (210, 235 nm) and 280 nm assigned to ¶–¶* and n–¶* transitions, respectively, in the electronic spectrum of the ligand. These transitions also found in the spectra of the complexes, but they are shifted towards lower wavelength, confirming the coordination of the ligand to the metallic ions. The first band around 210 and 235 nm is probably due to a ¶–¶* of the exocyclic bond in heterocyclic ring and also assigned to the two carbonyl groups. However, the second band around 280 nm is due to presence of COOH group[12].

In case of orotate complexes, the carboxylic group is blue shifted with increase the intensity (absorbance). This result confirms the complexation of metal ions via carboxylic group (monodentate chelating). The complex has a shoulder broad band at 325 nm may be assigned to the following d–d transition. The following complexes showed the absorbance values around 280 to 325 nm may be assigned to the following d–d transition. This result confirms the complexation of metal ions via carboxylic group.

ANTI MICROBIAL SCREENING

Experiment

Materials Used

- MH agar
- Petri plates
- Autoclave
- Conical flasks 250ml
- Laminar air flow
- Required organisms

SAMPLE PREPARATION

The required number of MH agar plates was prepared. The compound 40mg/ml is made in DMSO. And discs are soaked 40 µl in them and dried. The required organisms (S. aureus, Candida albicans, Pseudomonas aurginosa) are made ready in nutrient broth in proper dilution such that 10^8 cells/ml.0.1ml of the organisms in the broth is evenly spreaded in the labelled petri plates. The discs are impregnated on the plates. With the disc with compound C 1 and C 2 and Negative

control DMSO, Positive control antibiotic discs. Plates were incubated at 37^{o}C for 18 -24hrs, and zones of inhibition were measured.

RESULTS AND DISCUSSIONS

The ligands and their metal complexes were screened against bacteria and fungi. Antibacterial screening was done by the Disc diffusion method. The bacterial organisms used are *Pseudomonas aeruginosa* (gram +ve) and *Staphylococcus aureus* (gram +ve). Cultures of test organisms were maintained in nutrient agar media and subcultured in Petri dishes prior to testing. The fungal organism used is *Candida albicans.* Cultures were maintained on potato dextrose agar slants and subcultured in petri dishes prior to testing. The agar disk diffusion method was employed for the determination of antimicrobial capacities of the metal complexes. The test microbes represented the standard strains of P. aeruginosa and S. aureus as Gram-positive bacteria; and the fungi: Candida albicans. The antibiotics; Amphotricin and Amphicillin were used as standard references for Gram-positive bacteria and fungi, respectively. The metal complexes individually exhibited varying degrees of inhibitory on the growth of the organisms tested. This may attributed to the nature of the ligands, the nature of the metal ions, and the combined effect of the metal and the ligand [13]. The % activity index data indicate that the ligands show lower activity than their metal complexes shows the highest activity against all tested microbes, in addition, the activity follows the following order:

C_1: P.aurginosa > S.aureus

C_2:P.aurginosa > S.aureus

For fungal activity (C. albicans)

$C_2 > C_1$

The following complexes were tested for antimicrobial screening. The results were satisfactory. They have shown nearly similar inhibition activity like standard antibiotics against standard microbes.

C1 is Nickel- Orotic- imidazole

C2 is Nickel-Dafone –thiocynate[14]

For comparison, the metal-free orotic acid and its metal complexes, were tested for their MIC values. The results are presented in Table 3 as minimum inhibitor concentration(MIC) values, which is the minimum concentrationto inhibit the growth of bacteria or fungi[15].

[14] **Synthesis of $Ni(dafone)_2(NCS)_2$**

$Ni(ClO_4)_2(H_2O)_6$ (0.370 g, 1.00 mmol) was dissolved in water and taken in a long tube. Methanolic solution of dafone (0.182 g, 1.00 mmol) was added slowly to the above solution. Methanolic solution of KSCN (0.097 g, 1.0 mmol) was added slowly to the top the above solution and was kept for crystallization at 5° C. After two days green coloured crystals were separated. Yield 0.270 g (0.501 mmol, 50 %). Anal. Cald. $C_{24}H_{12}N_6NiO_2S_2$ (M.Wt. 539.23) C, 53.46; H, 2.24; N, 15.58. Found: C, 53.35; H, 1.69; N, 15.22. Important IR absorptions (KBr disk, cm^{-1}): 2081, 1809, 1738, 1597, 1292, 839, 534 and 476.

Table 3: Antimicrobial Results

S. No.	Organism		Zone of Inhibition in mm			
		Orotic Acid	C1	C2	Amphotricin B	Ampicillin
1	S. aureus	10	10	-	-	10
2	Pseudomonas aeruginosa	10	20	12	-	nil
3	Candida albicans	20	12	13	12	-

CONCLUSIONS

We presented the results of the synthesis and characterization studies of a series of mixed-ligand complexes involving orotate and imidazole ligands. Among these complexes $Ni(HOr)(imd)_2(H_2O)_2$ and $Ni(dafone)(NCS)_2$ were tested for antimicrobial properties. The IR spectra reveals the existance of functional groups and coordinated pseudohalide ions. The UV spectra confirms the complexation of metal and ligand. The particular absorbance values represents d-d transition of the metal complex. The bioactivity of these synthesized compounds is a complex phenomenon related to different factors and the metal complexes are more active than the free ligands. These active compounds may serve as a starting point for further studies on metal complexes acting as drugs.

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